

# Chapter 28

## Regulatory Aspects of Grape Viruses and Virus Diseases: Certification, Quarantine, and Harmonization

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**Abstract** While the existence of grapevine diseases and their negative impacts on vegetative growth and fruit production and quality have long been acknowledged, it is only relatively recently that clean stock programs around the world have made a significant impact on the health status of grapevines by providing the highest-quality virus-tested propagation material. Clean stock programs strive to ensure that continual advancements are made in disease detection and elimination while working with government agencies to develop and update certification and quarantine regulations. Global efforts to unify regulations among trading partners are necessary to ensure grapevine health worldwide.

**Keywords** Certification • Quarantine clean stock • Disease • Grapevine • Regulatory programs • Sanitary selection • Virus

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## Introduction

Various centers of expertise around the world use detection and therapy techniques (Rowhani et al., Chap. 20; Blouin et al., Chap. 21; Rowhani, et al., Chap. 22; and Golino et al., Chap. 27 in this book) to create grapevine selections that test negative for damaging viruses. The quality of the clean products depends upon the resources available to those centers and the goals of the individual programs. Producing truly virus-free planting stocks is a lofty goal; therefore, experts make practical judgments about how to use finite resources to make the most meaningful improvements possible in vine health. Some of the challenges faced by these expert centers involve making judgments about (1) which virus(es) has economically significant effects on vine health, (2) whether some viruses can be tolerated, (3) whether a virus can be practically excluded in a given geographic location once clean material is delivered to nurseries, (4) how to adopt the latest diagnostic advancements to streamline to production of clean stocks, and (5) how to prevent the infection of clean stocks in foundation vineyards. When it comes to vine health vis-à-vis virus testing and treatment, progress is made over time, with improvements in diagnostic and therapeutic technologies and with the education of scientists, regulators, nurseries, and growers. In some ways, it is analogous to the continuing work of specialists in human health, i.e., there is never a perfect control of the grapevine virus diseases through clean plant programs, but there are steady and measurable improvements where the programs are well funded and endorsed by the grape and wine community.

In an effort to document the benefits of the California's certification program and the impact on vine health, a survey of North Coast California vineyards was conducted. Each vineyard was classified by age and a subset of vines was tested for nine different viruses, all of which have been detected in California. The prevalence of each virus found in vineyards decreased steadily over the decades when the viral profiles were compared. The only virus, which was not significantly reduced since the program was established in the 1950s, was *Grapevine rupestris stem pitting-associated virus* (GRSPaV), which is notoriously difficult to eliminate. In fact, this virus is exempted from the California and many other certification programs because, despite numerous efforts, the effect of GRSPaV on vine performance has not been established, and it may be pollen transmissible (Rowhani et al. 2000), which means it would be challenging to control. Many of the other viruses common in older vineyards (planted from 1880 to 1980) were not found at all in the younger vineyards sampled (planted from 2011 to 2014). The two exceptions were *Grapevine leafroll-associated virus 3* (GLRaV-3) and *Grapevine red blotch-associated virus* (GRBaV), both of which are spread by vectors in this part of the state (Arnold et al. [in press](#)). The data show a steady reduction in the incidence of regulated virus diseases in newer vineyards with major challenges still posed by the viruses which are readily transmitted by vectors. To our knowledge, this is the first comprehensive study of the benefits of a certification program in terms of the prevalence of viruses in commercial vineyards

Here, we outline certification and quarantine programs in some of the major grape-producing areas of the world and discuss harmonization efforts. Our intent is to provide a realistic account of the different programs while suggesting opportunities for improvement.

## **Certification**

Certification is defined as the confirmation of certain characteristics of an object, person, or organization. In plant pathology, it usually involves a product certification, which requires processes intended to ensure that a product meets certain quality standards, i.e., freedom from a pathogen. In the very specific case of grapevine certification, it normally involves viruses combined with cultivar and/or clonal identification, and often viticultural performance. Discussions have also been initiated in various quarters of certification programs to consider freedom from the crown gall bacterium *Agrobacterium vitis* and from the fungi which cause trunk diseases. Where crown gall is part of an existing program, detection is often based on visual observation of symptoms.

Certification programs are usually, but not always, performed at public institutions with the involvement of state, national, or regional regulatory officials. Grapevine certification programs worldwide share the common goal of providing what is called foundation stock (G1 stock in international regulatory terminology). We will discuss some of the various programs and the procedures and protocols which are used by each.

### ***The European Union***

There is over a century of experience in grapevine selection (for both clonal and sanitary purposes) in the European Union (EU). This process had its beginning with efforts initiated in Germany near the end of the nineteenth century. The commitment to improve grapevine sanitary conditions grew stronger in the 1960s as a consequence of two events: first, the development of new knowledge on the viral nature of some serious disorders, such as fanleaf degeneration, and the potential to procure virus-free shoot tips from infected plants through thermotherapy; and second, the alarming sanitary deterioration of the crop in many regions, which was in part counterbalanced by the programs of individual member states (France, Germany, and Italy). These events prompted the EU Council to issue directives for the improvement of the EU's viticulture industry. The first Directive 68/193 (issued on 9 April 1968) on the "Marketing of Vegetatively Propagated Material of Grapevines" categorized propagative materials as "basic," "certified," and "standard" and defined the sanitary characteristics of mother vineyards destined for their production.

Whereas participation in the California certification system (and other North American programs) is voluntary, in Europe it is mandatory. Even so, in the European program, the acceptance of the “standard” category allows for the propagation of materials lacking significant phytosanitary guarantees. In reality, the “standard” category was a consequence of the lack of clones suitable for certification when the program was initiated and of the lengthy and costly procedures required for their procurement and increase. However, the same directive contained the auspices for a progressive improvement of the sanitary status toward production of “certified” materials. Enforcement toward that endeavor came in Directive 2002/11 (14 February 2002), issued to modify Directive 68/193, which abolished the production of rootstocks in the “standard” category (which had already been required by some member countries) starting January 2005.

Moreover, EU certification schemes should not be regarded merely as clean stock programs applied to viticulturally uncontrolled mother sources. Registration and certification of source materials require identification as true-to-type and verified as to clonal origin, as well as documented viticultural and oenological performance. The identification of clones is regulated by EU Directive 72/169 and outlined in a Resolution of the Office International de la Vigne et du Vin (Anonymous 1991). Thus, in the EU, clonal and sanitary selection involves interdisciplinary activity requiring the joint effort of viticulturists, virologists, and wine grape technologists.

The Directive 68/193, successively modified and integrated by the Directives 71/140 and 77/629, was an innovative regulation of great social and economic importance because it included concepts for certification and sanitary status accepted as valid in all EU member countries. It defines the sanitary requirement of current EU certification as follows: “In the vineyards producing basic material, harmful virus diseases, notably fanleaf and leafroll, must be eliminated. Vineyards producing materials of other categories must be kept free from plants showing symptoms of virus diseases.” By-laws generated by these directives were promulgated in EU member countries and national certification schemes were implemented.

It is clear that the sanitary provisions of these directives are not satisfactory and fail to ensure an acceptable sanitary status of propagative material in any category. Furthermore, they are no longer adequate, failing to take into account recent scientific discoveries in grapevine virology. Much progress has been made in the etiology, epidemiology, and diagnosis of grapevine viruses and virus-like diseases; unfortunately, the improved technology is largely ignored. In an attempt to address this issue, some European countries enacted their own measures. Although national certification schemes implemented in EU member countries are inspired by, and more or less conform to, EU directives, they vary. Some differences pertain to the viticultural aspects of clonal selection. In Germany, for example, the selection process requires a longer time period compared to France and Italy. Additional differences reside in sanitary requirements where, in most instances, they are stricter than those in the EU directives, the latter requiring tests for only fanleaf and leafroll diseases.

Overall, individual sanitary schemes may vary widely, with Portugal, France, and Italy requiring all selections to test negative for rugose wood diseases, which is not required in Germany. In Spain, assays are limited to rupestris stem pitting and corky bark. And, interestingly, EU directives require that all rootstock be free of fleck, but scion sources are exempt from this requirement.

The abovementioned scenario has impeded the full harmonization of the European production scheme, a goal attempted by Directive 68/193. As a consequence of continued efforts involving several organizations and grapevine virologists for an updated directive to harmonize the system, the EU has developed a new Directive 2002/11/CE. However, the practical impact of this directive depends greatly on the contents of the technical appendix, which has yet to be made public for a full evaluation of its content.

Although other differences exist in how certified materials are maintained, propagated, and distributed, certain steps are commonplace to all schemes. Registered clones (primary sources or nuclear stocks) are maintained by the organization or individual who owns them (“obteneur” or conservation breeder) and undergo a first multiplication in specialized facilities. The propagating material is distributed to nurseries for the establishment of certified mother blocks and used for the production of certified budwood, rooted cuttings, or grafted plants for commercial plantings. An officially authorized organization is responsible for the sanitary status, the origin of materials, and quantities of certified plants produced prior to being issued certification labels.

## *California*

In the New World, the oldest grapevine certification program is the California Department of Food and Agriculture (CDFA) Grapevine Registration and Certification (R&C) Program, established in 1956 (Alley and Golino 2000). Other state programs in the United States are modeled after it, and several use its stock as initial source materials for their own certification or clean stock programs. The California program also has historic ties to clean stock programs of Canada and Australia, where similar techniques and protocols have been adopted.

The current certification program is described in some detail to illustrate typical protocols used for grapevine clean stock programs. In two key areas, this program differs from many European programs. First, the program is entirely voluntary; nurseries are free to participate or not under this system. Second, this program does not include cultivar or clonal evaluation – a key part of many European grapevine clean stock programs (Golino and Wolpert 2003). Selections entering the program are normally chosen for potential viticultural merit and become available for performance evaluations once the phytosanitary requirements of the program are met. These efforts are managed separately from the regulatory program, which focuses on target diseases and pathogens coupled with management protocols intended to reduce the chance of reinfection of clean stock.

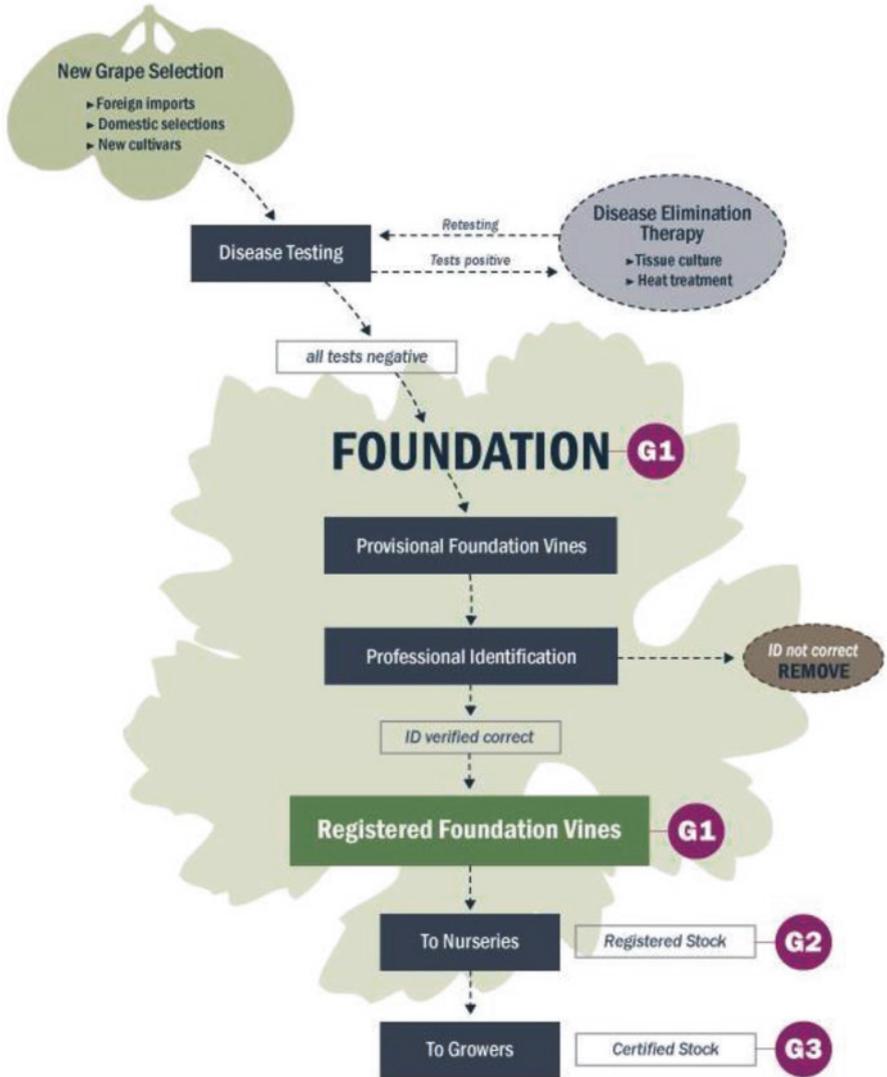
Foundation Plant Services (FPS) is a service department in the College of Agricultural and Environmental Sciences at the University of California, Davis. This department performs the disease testing required by the CDFA R&C program and maintains the foundation vineyards that supply nursery participants.

Each grapevine in the FPS collection is assigned a registration category according to its health and cultivar identification status. Registered foundation grapevines are those propagated from sources that meet requirements mandated by the CDFA R&C program guidelines, and professionally identified to cultivar. Provisional foundation grapevines are those that have passed the disease tests (CDFA R&C program), but cultivar identification is incomplete. Non-registered grapevines have either tested positive for targeted diseases, are of unknown disease status, or are of questionable cultivar identity. All non-registered grapevines are maintained separated from the elite foundation block, awaiting therapy or identification. Provisional and non-registered selections are distributed only to customers receiving notification of the grapevines' status and willing to assume all of the associated risks.

The CDFA R&C program includes provisions for three levels of grapevine stock: foundation, registered, and certified (Fig. 28.1). Propagation materials derived from foundation grapevines in the FPS foundation vineyard are known as "foundation stock." Participants in the CDFA R&C program use the California foundation stock to establish their own vineyards, referred to as "primary" or "secondary increase blocks." These blocks are inspected annually and are virus tested as required by CDFA inspectors. Cuttings taken from the registered increase blocks are used to produce "registered stock." Grape plants produced by own-rooting registered stocks or by grafting registered stock scions to registered stock rootstocks qualify as "certified stock." These plants are sold for commercial plantings. CDFA regulations govern all Grapevine R&C Program responsibilities, which include eligibility requirements, planting and maintenance requirements, inspection and testing procedures, and approval, suspension, and cancelation of certification, application, and fees.

All woody and herbaceous index tests prescribed in CDFA R&C regulations are used to screen grape materials before qualifying as foundation stock at FPS (Rowhani et al. 2005). In addition to the biological indexing (Rowhani, et al., Chap. 20 in this book), enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) are performed at FPS on every accession destined for inclusion in the foundation vineyard (Blouin et al., Chap. 21 and Rowhani, et al., Chap. 22 in this book).

Furthermore, during their first two growing seasons, the new accessions in the foundation vineyard are tested twice by ELISA for Grapevine leafroll-associated viruses; three nematode-transmitted viruses, namely, *Arabidopsis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), and *Tomato ringspot virus* (ToRSV); plus, *Grapevine fleckvirus* (GFkV) and *Grapevine virus A* (GVA). Thereafter, one-fifth of the foundation grapevines (third-leaf and older) are ELISA tested each year so that over a 5-year period the entire foundation vineyard is retested. This 20% retesting procedure is repeated for the life of the foundation vineyard. As an additional precaution at FPS, the foundation grapevines are periodically re-indexed biologically and retested by ELISA and PCR. Lastly, the foundation vineyards are visually



**Fig. 28.1** Flowchart of grapevines from introduction through foundation, registration, and certification

inspected twice per year, spring and summer, for disease symptoms. When suspicious symptoms are found, the grapevine(s) are immediately tested with laboratory-based assays and, as warranted, by woody host indexing. Infected grapevines are removed.

## *New York*

A grapevine certification program managed by the Department of Agriculture and Markets was established in New York State in the early 1960s. This program mirrored the program created earlier in California. The New York program was well perceived until the late 1980s when its attractiveness vanished. This coincided with an unprecedented expansion of the grape acreage in the State, which resulted, as expected, in the deterioration of the sanitary status of local vineyards. The recurrent issues of viruses in local vineyards and early accomplishments of the National Clean Plant Network (NCPN) for grapes triggered a strong grassroots movement led by local nurseries and the wine and grape industry to reinstate a certification program in New York State. The new program focuses exclusively on G2 blocks for which clean vines are primarily sourced from recognized foundation G1 blocks and strict site selection criteria are applied. Plants at each G2 block are visually inspected for viral disease symptoms in the spring and fall, and one-fourth of them is tested each year for viruses [GFLV, ToRSV, *Tobacco ringspot virus* (TRSV), GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, and GRBaV] by ELISA and/or PCR-based assays for the entire life span of the vineyard. If a vine is found infected, it is immediately removed and so are adjacent vines, whether infected or not.

## *Canada*

The Canadian Grapevine Export Program (CGEP) is managed by the Canadian Food Inspection Agency (CFIA) under Directive D-15-02. This directive replaces Directive D-97-06 to incorporate a systems approach for producing virus-tested grapevines for export certification to the USA. The material produced in this program can also be used within Canada. Independent components such as virus testing, field inspection, isolation distance, and vector control are considered to minimize the presence and spread of viruses. Plants produced under the CGEP must originate from first-generation (G1) stock produced by the CFIA or approved facilities, and these original mother plants are tested for viruses and virus-like pathogens of concern determined by the North American Plant Protection Organization (NAPPO) Standard No. 35. A combination of tests is performed on all G1 accessions, including biological indexing (woody and herbaceous), serological tests (ELISA), and PCR assays. Canada follows the G-level naming system where at each stage of propagation, progeny plants drop to a lower certification level. Plants qualifying for CGEP purposes belong to one of four categories (G1A, G2, G3, and G4) derived from G1 stock (Anonymous 2016b).

## ***Argentina and Chile***

Certification procedures with the characteristics of clean stock programs were established in Argentina in 2001 and in Chile in 2007. In both countries, nuclear stocks, which do not undergo clonal selection, are certified for the absence of a limited number of viruses. In Argentina, these are GFLV, Grapevine leafroll-associated viruses (GLRaV-1, GLRaV-2, and GLRaV-3), GFKV, rugose wood, vein mosaic, and vein necrosis. In Chile, they are GFLV; GLRaV-1, GLRaV-2, and GLRaV-3; GVA, and *Grapevine virus B* (GVB) (Golino et al. 2015).

## ***South Africa***

The Vine Improvement Association in South Africa was established in 1986 to promote the interests of the South African wine industry through plant improvement and certification of vine propagation material. The certification scheme for wine grapes was licensed under the South African Plant Certification Scheme for Wine Grapes under the Plant Improvement Act No. 53 of 1976 by the South African Department of Agriculture, Forestry and Fisheries. The South African certification protocol encompasses clonal selection and requires rootstock and scion material to be free from the following virus diseases: fanleaf, fleck, leafroll, corky bark, stem pitting/grooving, and “Shiraz disease” (unknown etiological agent). It also requires visual freedom from crown gall (*Agrobacterium vitis*), bacterial blight (*Xylophilus ampelinus*), *Pythium* spp., *Phytophthora* spp., and a group of nematodes and insects among which are the pseudococcid mealybugs (*Pseudococcus longispinus* and *Planococcus ficus*), known vectors of some viruses associated with leafroll and rugose wood (Anonymous 2009b; Golino et al. 2015).

## ***New Zealand and Australia***

In an effort to manage the quality of new grapevine plantings in New Zealand, the New Zealand Winegrowers Board has developed a Grafted Grapevine Standards and an associated certification program with the objective of minimizing the probability of infected material being released to the industry. Certified mother plants are true-to-type at the varietal level and free from GLRaV-3 at the time of testing. Specific physical specifications and the related management systems are within the scope of the requirements (Anonymous 2011).

The Australian Standard for Grapevine Propagation Material is a clean stock program announced with the publication “AS5588–2013 Grapevine Propagation Material.” As specified, the standard encompasses the definition of specifications and guidelines related to the health status; origin, source, and traceability; and the

authentic, valid naming of grapevine propagation material (Hayes 2013). Certification is entirely voluntary in Australia. Certified vines must test negative for GLRaV-1, GLRaV-2, GLRaV-3, and GLRaV-4 and GFkV, GVA, GVB, and GRSPaV. Technically, corky bark disease is screened at the border in quarantine; the lab testing for GVB is considered separate from that quarantine screening which may reflect some redundancy since there is wide acceptance that GVB is closely associated with corky bark disease. Schemes may undertake active testing for some or all these viruses and possibly some other pathogens, certainly at the nuclear stage. At the mother block stage, they may undertake active testing and/or visual observation (F. Constable, personal communication).

## Quarantine

Quarantine is defined as official confinement of regulated articles for observation and research or for further inspection, testing, and treatment (Martin and Tzanetakis 2014). Quarantine regulations restrict the movement of grape nursery stock into most countries. These regulations attempt to prevent the importation of exotic pests and pathogens into pest-free areas and to limit the distribution of economically important pests and pathogens that might be under domestic control programs. Quarantine regulations for *Vitis* are highly variable between countries. Some of the reasons are historical, but in general, new grape-growing regions have fewer disease and insect problems than the older grape-growing regions. These new regions are more likely to attempt to protect their industry from the inadvertent introductions of exotic pest problems from older grape-growing regions. Some of the strictest regulations in the world for *Vitis* are found in Australia, Chile, New Zealand, South Africa, and the USA; in these countries importation may take years. However, the relative health and freedom from pests that vineyards enjoy in these regions are a reasonable compensation in the eyes of most viticulturists. Another factor faced by international traders in grape plant materials, beyond the variation in the regulations themselves, is the uneven enforcement of existing regulations. Two countries might, in theory, have identical regulations when, in fact, grape nursery stock would move freely into one country and the identical stock could not enter the second country (Golino 2000).

## *The European Union*

The EU adopted a very strict regulation concerning the introduction of grapevine germplasm from areas outside it. According to Directive 2000/68, the importation of plants or plant parts (except fruits) of *Vitis* spp. is forbidden. However, under certain conditions, member countries can allow the importation of germplasm after its testing and indexing under strict quarantine conditions. For example, an Italian

legislation (DM 31.01.1996), in agreement with the European directives, requires the following procedure concerning *Blueberry leaf mottle virus* (BLMoV), the grapevine yellows phytoplasmas (including that causing Flavescence dorée), *Peach rosette mosaic virus* (PRMV), TRSV, ToRSV, *Xylella fastidiosa*, *Xylophilus ampelinus*, Ajinashika disease, Grapevine stunt, and summer mottle. First, therapeutic treatments must be completed according to the technical guidelines FAO/IBPGR (International Board for Plant Genetic Resources), to be followed by indexing and laboratory analysis in proper facilities (Frison and Ikin 1991).

The sanitary provisions of current EU directives for non-quarantine pathogens are currently outdated and do not provide an acceptable status of propagative material in any category of plants, failing to consider and incorporate recent developments in grapevine virus disease research. Moreover, they lack guidelines for the implementation of sound research-based protocols. Under current directives, there is variation in the sanitary status of grapevine propagative materials produced in the EU; quality standards are largely determined by different national certification schemes. Even among the six member countries with large viticulture interests (Italy, France, Germany, Greece, Portugal, and Spain), the harmonization of sanitary protocols is lacking.

### *United States*

The Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) 7 Code of Federal Regulations (CFR) 319–37 governs the importation of propagative material into the USA. Currently, PPQ is revising 319–37 and other regulations governing the importation of plants for planting. A principal component of this revision is establishing a regulatory systems approach protocol, utilizing performance-based criteria. The criteria in the Grapevine Standard will be used as a basis for this regulation change. However, the legality of using performance-based criteria needs to be determined for this application. Actual implementation of the criteria outlined in the standard will be based on bilateral negotiations and result in the development of an acceptable operational work plan for the production and export of certified plants. The United States currently has grapevine, pome, and stone fruit trees certification programs, which have been approved by PPQ and implemented by interested states. These programs already meet most of the criteria outlined in the standard. However, PPQ needs to establish National Plant Protection Office (NPPO) oversight and minimum conditions for certification as outlined in the standard. The United States is currently piloting a national certification program through the NCPN for grapes with voluntary state participation through a memorandum of understanding (MOU). The certification standards under NCPN would meet the criteria outlined in this standard. At this point APHIS has the authority to legally implement the conditions of the standard (Anonymous 2009a). NCPN is a collaborative effort among three USDA agencies: APHIS for quarantine and regulatory programs, USDA-ARS for technology and germplasm issues, and the National

Institute for Food and Agriculture (NIFA) for outreach and partnership initiatives. Some NCPN Clean Plant Centers (CPCs) serve as quarantine centers (Gergerich et al. 2015). While NCPN has adopted the suggested G1–G4 terminology, the use of the tiered generation level concept has not been universally adopted.

## *Canada*

Canada regulates the importation of grapevines from all countries according to the guidelines outlined in Canadian Food Inspection Agency (CFIA) Directive D-94-34: Import Requirements for Grapevine Propagative Material (Anonymous 2014). Canada allows the importation of grapevine propagative material that has been certified under certain US state certification programs, including those in California, Oregon, and Washington. Currently, France and Germany are the only off-continent sources with CFIA-approved nurseries certified to export specific, approved grapevine rootstocks and varieties/clones for propagation to Canada. Any previously non-approved grapevine rootstocks and varieties/clones that originate from a country other than the United States and that are produced by a non-approved nursery must be authorized by the CFIA prior to importation, even if the material originated from within a country or certification program for which other materials have previously been approved by the CFIA. All grapevine material from CFIA-approved foreign sources must be free of quarantine and regulated non-quarantine pests of Canada and must be free from soil, sand, and related plant debris. Grapevines imported from Europe must be hot water treated for phytoplasmas. For grapevines originating from the United States and destined to British Columbia (BC), approved treatments for the control of phylloxera and virus-vectoring nematodes that are not known to occur in BC must be applied. A phytosanitary certificate must accompany each consignment. The approved certifying authority of the exporting country must provide Canada with separate certificates of origin for the mother blocks of the imported material for every shipment. Furthermore, they must ensure that certification tags clearly indicating the source and its applicable certification code are attached to each lot of grapevines exported to Canada. Upon arrival at the first point of entry in Canada, all shipments are subject to inspection, including verification of documentation, by CFIA. Each year, a sampling of selected material imported from CFIA-approved sources is collected and sent to the CFIA Sidney Laboratory for testing. The detection of quarantine and regulated non-quarantine pests will lead to a reevaluation of the program and possible suspension of approved rootstock and variety/clones, nurseries, or the program.

Grapevine propagative material from any CFIA non-approved foreign source may only be imported into Canada under Section 43 of the Plant Protection Regulations. In this case, imported material must be sent directly to the CFIA Sidney Laboratory for post-entry quarantine and full-range testing before being released.

## *New Zealand and Australia*

The Ministry for Primary Industries (MPI) is charged with leadership of the New Zealand biosecurity system. *Vitis* dormant cuttings and plants in tissue culture are approved for entry into the country following guidelines set forth in the MPI Standard 155.02.06 Importation of Nursery Stock pursuant to section 24A of the Biosecurity Act of 1993. Imports must be accompanied by a phytosanitary certificate and follow approved inspection, testing, and treatment requirements for insects, mites, fungi, bacteria, viruses, viroids, phytoplasmas, and diseases of unknown etiology (Anonymous 2016a).

In Australia, economically important pests and pathogens not currently present in Australia are “quarantinable,” and before release, all imported planting material must be determined by the Australian Quarantine and Inspection Service (AQIS) to have non-detectable levels of these pathogens/pests. At present, AQIS is not required to test for endemic pathogens in planting material entering the country. As a consequence, material being released from quarantine is not usually of a defined health status (Constable and Drew 2004).

## **International Phytosanitary Systems**

In 1995, the World Trade Organization (WTO) was formed and member nations agreed to honor its charter. Included in these international codes is an Agreement on the Application of Sanitary and Phytosanitary Measures, known as the SPS Agreement. This agreement contains provisions allowing member nations to enforce regulations that might otherwise violate the terms of free trade when those provisions are “necessary to protect human, animal or plant life or health.” It is under these provisions that most national importation and quarantine regulations are allowed. It is incumbent on member states to ensure that these restrictions are not disguised trade barriers. The WTO administers disputes and provides resolutions in cases where member countries feel that the SPS agreement is being used unfairly or arbitrarily (Kreith and Golino 2003).

The WTO/SPS authorizes the setting of international trade standards. In cases involving plant health, the Food and Agriculture Organization (FAO) of the United Nations is the responsible organization, working under the terms of the International Plant Protection Convention (IPPC; <https://www.ippc.int/en/>), a treaty, which began in 1951. As of September 2015, the Convention had 182 signatories. The IPPC has three core areas: international standard setting, information exchange, and implementation of IPPC phytosanitary standards. At this time, the process involves establishing guidelines and definitions, as well as coordinating efforts of regional plant protection organizations to establish consistent standards. The regional plant protection organizations are being asked to make the first efforts at harmonized standards, because geographically contiguous areas often share common exotic pest and

pathogen concerns and often work in concert to establish both internal and external control programs.

According to international guidelines, the national regulation programs – either through mandatory certification programs or official control programs for target diseases for each commodity – could allow classification of endemic economically important diseases as regulated non-quarantine pests. Regional, national, state, or local regulations might also serve this purpose. By establishing strict regulations, only imported nursery stock meeting high standards of freedom from specific domestic diseases could enter the regulated area.

NAPPO, operating under IPPC guidelines, recommends that the certification program have clearly defined certification levels, including nomenclature, propagation, and pest management measures (Anonymous 2002). According to NAPPO guidelines, certification levels represent successive generations of propagation material from the original tested material and may have additional phytosanitary measures applied depending on the generation (Anonymous 2004). As such, they can represent a categorical measure of the health status of certified plants. The generation or “G-level” concept is used to identify the degree to which plant stock is related to the original virus-tested plant material (Fig. 28.1). Regulations developed by certification programs specify the conditions under which each generation level must be maintained in order to qualify for the program. In vegetatively propagated crops, G1 material refers to the original mother plants which have tested negative for all targeted pathogens and is the source for all further propagation; it is normally housed at a CPC. Authors have used a myriad of terms to describe G1 material, including foundation, nuclear, elite, pre-elite, extra super elite, pre-prebasic, and pre-selection (Martin and Tzanetakis 2014; Boidron 1995). Generation 2 (G2) material is propagated from G1 stock and is frequently maintained by nurseries in increase blocks to supply to commercial growers. G2 stock may also be known as elite, foundation, super elite, or pre-basic. Generation 3 (G3) material is propagated from G2 stock; it is commonly used in secondary increase blocks and certified nursery blocks. G3 stock is also known as registered, basic, elite, and increase block. Generation 4 (G4) plant material is propagated from G2 or G3 stock. G4 stock refers to the certified plants delivered to consumers (Anonymous 2012; Martin and Tzanetakis 2014).

## Efforts at Harmonization

The NAPPO is a regional organization with members from the national plant protection organizations of Canada, the USA, and Mexico. It is one of many regional organizations whose primary responsibility is to develop regional plant protection standards, designed to protect the member states from the entry and establishment of pests, while facilitating trade. In addition, NAPPO participates with other regional plant protection groups within both the Western Hemisphere and the global level to develop international standards. The document “Guidelines for the Importation of

Grapevines into a NAPPO Member Country RSPM #15 Part 1: Viruses and Virus-like Pests, Viroids, Phytoplasmas, and Bacteria” was developed by a committee of experts and signed on October 20, 2002. This document is the initial regional guideline for the development of harmonized North American Standards for grapevine nursery stock (Anonymous 2004); it is superseded by RSPM 35: “Guidelines for the Movement of Stone and Pome Fruit Trees and Grapevines into a NAPPO Member Country” signed on October 19, 2009.

In the area of grape certification, Canada has a formal national certification program, which is voluntary. Mexico has no national grape certification program, but the majority of grape nursery stock comes from California and must meet California certification standards to be imported. The United States operates under a system of voluntary state certification programs, which combined with strict quarantine regulations have resulted in high-quality grape nursery stock with a minimum of regulatory infrastructure. There is no national standard for grapevine nursery stock, though such a national standard is being developed under the United States Department of Agriculture (USDA)’s NCPN organized in 2009 as a result of the 2008 Farm Bill (Gergerich et al. 2015). However, as regional organizations like NAPPO and international agencies like the FAO work to harmonize standards for the movement of plant materials internationally, a more formal, coordinated national and international system is being discussed in the United States to ensure that growers and industry are protected from non-quarantine damaging diseases which can be carried in planting stocks.

## Challenges of Globalization and New Technology

One important result of globalization has been that there has been a dramatic increase in plant pathogens and pests presenting new challenges to the production and security of food, fiber, and forest resources (Bostock et al. 2014). One of the most effective pathways for the introduction of new biological agents has been trade in “plants for planting” because infected and infested seed and nursery plants provide a very robust avenue for the establishment of exotic pests and diseases. In the case of *V. vinifera*, movement of planting material around the world has been a very effective means of dispersal of many serious pests and disease (Golino 2000; Martelli, Chap. 2 in this book).

Challenges introduced by advances in technology must be considered as well. The application of high-throughput sequencing (HTS) analysis in grapevine virology has yielded significant achievements in the field including the discovery of the new viruses, the use as a superior routine diagnostic tool, and the most comprehensive detection technology for certifying the phytosanitary status of commercial grapevine propagation stocks. These successes are detailed in Chap. 30 (Saldarelli et al. of this book). However, there are limitations inherent with the benefits of this advanced technology. Bioinformatic analysis does not prove pathological causality, nor does it describe biological characteristics of a virus. In order to weed out

insignificant background viruses, biological effects of discovered viruses must be assessed. Studies including graft transmission, fulfillment of Koch's postulates, and spread and distribution analysis can assess the agronomic significance of novel viruses. HTS is a powerful, advanced diagnostic tool that will yield the most meaningful results when coupled with basic plant pathological methods.

## Conclusive Remarks

The International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG) represents grapevine virologists worldwide. These scientists believe that concerns about the introduction of exotic viruses and virus diseases to nonindigenous regions should be a primary focus of international regulations governing the movement of grapevine nursery stock. At the same, this group acknowledges the need to maintain diverse heritage grapevine cultivars and clones in regional areas where the resources may not be available to sanitize all grapevine plant stocks.

The high value of grapevine plant material, the competitive nature of the grapevine nursery business, and rapid globalization of the grapevine and wine community have resulted in some fundamental changes in the availability of clean grapevine selections, cultivars, and clones. Recent years have seen a worldwide increase in patented grapevine cultivars and rootstock selections, the introduction of trademarked clones of traditional cultivars, and the development of proprietary programs in which valuable selections are marketed exclusively and cannot be obtained by all growers. Some sales contracts for grapevine nursery stock even limit the rights of growers to subsequently propagate selections. Governments are having increasing difficulties funding public programs for importation, certification, and distribution of grapevine stock, making the few remaining programs increasingly international in influence. The technology involved in both grapevine identification and plant disease detection has changed radically in the past decade and is likely to continue doing so. All of these factors have a profound effect on international grapevine quarantine, clean stock, and certification programs.

Continuing work on the development of harmonized international standards for grapevine nursery stock is likely to increase trade among the grape-growing regions of the world. This would result in more open competition for the nursery industries and lower prices for growers but would also increase the potential for importation of damaging pests and diseases and a resulting degradation of crop quality and productivity. As efforts are made to harmonize grapevine certification protocols, high standards are essential to ensure that no viticultural region is compromised by the introduction and spread of diseases. In the USA, the NCPN for grapes has the responsibility to produce the top tier (G1) plants that would serve as the starting material for state certification programs. There is also an effort to develop a grapevine certification standard that would be agreed to by all states producing certified stocks, essentially creating a national standard. These efforts include an attempt to

harmonize certification standards with trading partners (e.g., EPPO and/or NAPPO standards) where possible (Anonymous 2008). Within this context lies the challenge of determining which microbes are relevant and actionable from a regulatory point of view, in order to maintain the delicate balance of protecting local and national industries from introduced pests and diseases while reaping the benefits of expanded trade (Golino et al. 2015). International regulations are based on a philosophy of expediting a level playing field vis-à-vis trade. However, in practice, from a plant protection perspective, plants for planting are a potentially dangerous vehicle for the introduction of damaging pests and diseases, and our ability to accurately calculate risk is limited. So it is not surprising that in practice, local regulators tend to be very conservative about change and slow in modifying standards. For this reason, a continued dialogue between scientists, regulators, nurseries, and growers is crucial to provide the highest-quality virus-tested propagation material.

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